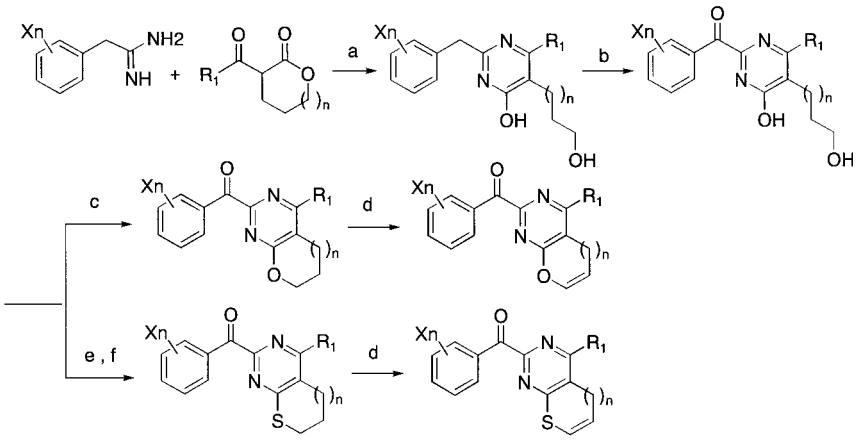

Figure 2. Synthetic methods for 2-benzoylpyrimidines with a 4,5-fused heterocyclic system.

transplanted rice and barnyardgrass. Compounds **22** and **17** were considered to be the best analogs with respect to excellent pre-emergence efficacy against troublesome weeds like giant foxtail, crabgrass and common lambsquarter in soybean fields as well as having good selectivity. These compounds could control barnyardgrass at low rates in pre- and early post emergence stage in paddy fields and showed long-lasting activity.

Elongation and cell division of treated plants were strongly inhibited by the benzoylpyrimidines and bulbous-shaped roots were also observed. Such phototoxic symptoms suggested that the mode of action was the same as with herbicidal dinitroanilines.[4]

The 2-benzoylpyrimidines shown above are a new class of herbicide with excellent activity against annual grass weeds and some broadleaved weeds in soybean and paddy fields.

REFERENCES

1 Kato T, Yamanaka H and Hiranuma H, Reaction of methylpyrimidine derivatives with isoamyl nitrite *Yakugaku Zassi* **90**:877–882 (1970).
2 Sakamoto T, Kanno S and Yamanaka H, Synthesis of pyrimidinyl ketones *Heterocycles* **6**:1616–1621.
3 Hermann CKF, Sachdeva YP and Wolfe JF, A new synthesis of aryl hetaryl ketones via SRN1 reactions of halogenated heterocycles with potassiophenylacetonitrile followed by phase-transfer catalyzed decyanation. *J. Heterocycle Chem* **24**:1061–1065 (1987).
4 Hackskaylo J and Amato VA, Effect of trifluralin on roots of corn and cotton *Weed Sci* **16**:513–515 (1968).

The mystery of the trichothecene 3-*O*-acetyltransferase gene: *Tri101* evolved independently of other trichothecene biosynthetic genes in the gene cluster

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Abstract: Trichothecene resistance is achieved *via* 3-*O*-acetylation on the biosynthetic pathway of deoxynivalenol in *Fusarium graminearum*. The responsible 3-*O*-acetyltransferase gene, *Tri101*, was located between the UTP-ammonia ligase gene and the phosphate permease gene, and not in the trichothecene biosynthetic gene cluster. As predicted by the presence of its homologues in yeasts, the resistance gene proved to have evolved independently of other biosynthetic genes.

In *Fusarium sporotrichioides*, *FsTri*101 (a functional homologue of *Tri101*) was also located these two 'house-keeping' genes. However, *FsTri101* appeared not to play a pivotal role for self-resistance, suggesting that other defensive options are the primary working strategies for the type A trichothecene producer.

Keywords: antibiotic resistance; *Fusarium* mycotoxin; gene cluster; independent evolution; trichothecene biosynthesis

Trichothecene mycotoxins are potent translation inhibitors of protein synthesis in eukaryotes and prevent polypeptide chain initiation/elongation by binding to 60S ribosomal subunits. We have shown that 3-*O*-acetylation of the ring works as a self-resistance mechanism for the t-type trichothecene producer *Fusarium graminearum* Schwabe F15. Based on this finding, the cDNA and cosmid clones for the related acetyltransferase gene, *Tri101*, have been isolated and characterized.[1] The *Tri101*-containing cosmid clones did not contain *Tri4*, *Tri5*, and *Tri6*, other trichothecene biosynthetic and regulatory genes isolated so far.[2]

Here we have sequenced the cosmids containing *Tri101* and found that this resistance gene is not

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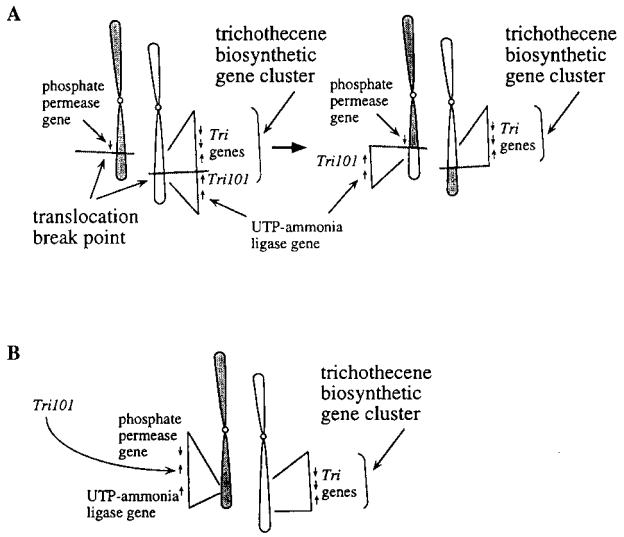

Figure 1. Two models to explain the isolated occurrence of *Tri101* from other trichothecene biosynthetic genes in the cluster. A. A model based on the reciprocal translocation. B. A model based on independent evolution of *Tri101*.

flanked by trichothecene biosynthetic genes; it was located between the UTP-ammonia ligase gene (upstream of *Tri101*) and the phosphate permease gene (downstream of *Tri101*), which were constitutively expressed and not related to trichothecene biosynthesis. To investigate whether the isolated occurrence of *Tri101* could be attributed to the reciprocal translocation of the chromosome containing the gene cluster (Fig 1A), we analyzed the non-producer *Fusarium* strains by Southern analysis. Genomic DNA of *Fusarium oxysporum* Schlecht. (ATCC60843) and *Fusarium equiseti* (Corda) Sacc (IFO31095) were digested with several rare-cutting restriction enzymes, separated by CHEF electrophoresis, and the blots were separately probed with these 'house-keeping' genes. Hybridization signals of exactly the same size were detected from each blot of genomic DNA digested with these restriction enzymes, suggesting that the UTP-ammonia ligase gene and the phosphate permease gene were also linked in *Fusarium* species that do not produce trichothecenes. The result indicates that the translocation event did not occur and that *Tri101* evolved independently of other biosynthetic genes in the gene cluster (Fig 1B). In support of this, homologues of *Tri101* have been found in wild-type *Saccharomyces cerevisiae* Meyer ex Hansen (GenBank accession no. Z73168) and *Schizosaccharomyces pombe* Lindner (AL023781), which are non-producers and sensitive to the toxins.

We next examined the genome of the type A trichothecene (see Fig. 2) producer *Fusarium sporotrichioides* Sherb (IFO9955). The type A producer has not been previously described producing a trichothecene with an acetyl group at the C-3 position, although the type B producers (such as *F graminearum*) are well able to do this.[3,4] Consistent with these observations, *F sporotrichioides* did not acetylate the C-3 position of T-2 toxin externally added to the culture. However, this strain also carried a functional 3-*O*-acetyltransferase gene (*FsTri101*) between the UTP-ammonia ligase gene and the phosphate permease gene, as in the case of the type B trichothecene producer. Northern analysis indicated that the mRNA level of *FsTri101* was not so high and was unaffected by the addition of trichothecenes, whereas expression of *Tri101* was strongly induced by sublethal levels of T-2 toxin

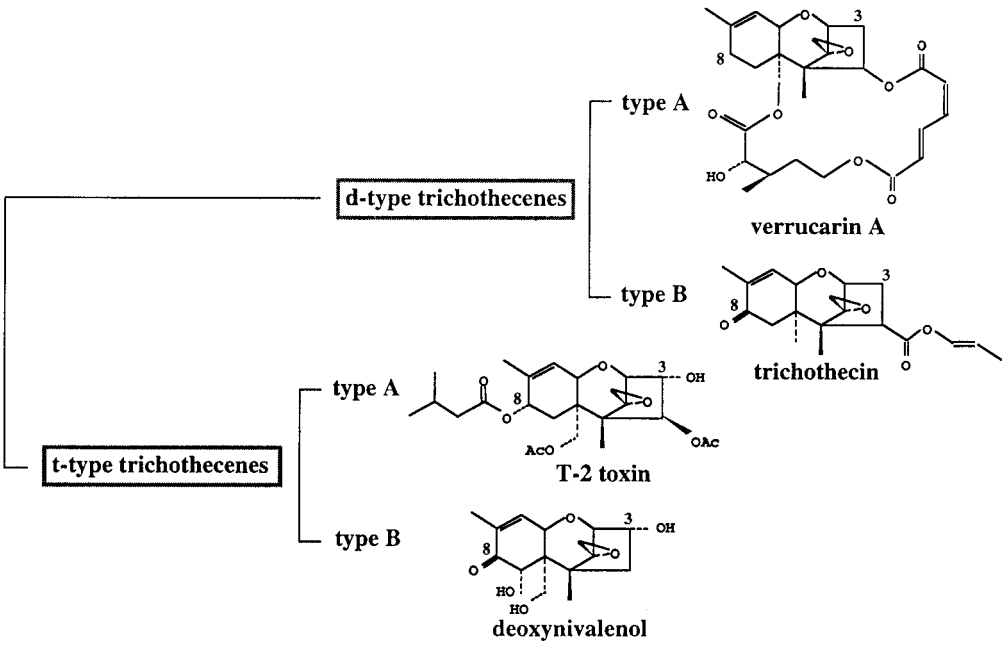

Figure 2. Structure and proposed classifications of trichothecenes. We previously classified trichothecenes by the presence (t-type) or absence (d-type) of the 3-hydroxyl group on the trichothecene ring.[1] They can also reasonably be divided into two types by the presence (type B; eg deoxynivalenol produced by *F graminearum*) or absence (type A; eg T-2 toxin produced by *F sporotrichioides*) of a keto group at the C-8 position.[9]

added to the culture.[5] These results suggest that the antibiotic modification is not necessarily a primary self-defence mechanism for all the t-type trichothecene producers, as we have previously discussed.[1]The type A trichothecene producer would have other defence options dispensable with 3-*O*-acetylation. These possible options might include modification or replacement of the drug target ribosome,[6] efficient efflux of the antibiotic by the membrane transporter,[7] and restriction of the membrane permeability against substances in the medium,[8] which might be substantially defective in the type B producer.

REFERENCES

1 Kimura M, Kaneko, I, Komiyama M., Takatsuki A, Koshino H, Yoneyama K and Yamaguchi I, Trichothecene 3-*O*-acetyltransferase protects both the producing organism and transformed yeast from related mycotoxins. Cloning and characterization of *Tri101*. *J Biol Chem* **273**:1654–1661 (1998).

2 Keller NP and Hohn TM, Metabolic pathway gene clusters in filamentous fungi *Fungal Genet Biol* **21**:17–29 (1997).

3 Yoshizawa T, Onomoto C and Morooka N, Microbial acetyl conjugation of T-2 toxin and its derivatives. *Appl Environ Microbiol* **39**:962–964 (1980).

4 Plattner RD, Tjarks LW and Beremand MN, Trichothecenes accumulated in liquid culture of a mutant of *Fusarium sporotrichiodes* NRRL 3299. *Appl Environ Microbiol* **55**:2190–2194 (1989).

5 Kimura M, Shingu Y, Yoneyama K and Yamaguchi I, Features of *Tri101*, the trichothecene 3-*O*-acetyltransferase gene, related to the self-defense mechanism in *Fusarium graminearum*. *Biosci Biotechnol Biochem* **62**:1033–1036 (1998).

6 Igresias M and Ballesta JP, Mechanism of resistance to the antibiotic trichothecin in the producing fungi. *Eur J Biochem* **223**:447–453 (1994).

7 Gottesman MM and Pastan I, The multidrug transporter, a double-edged sword. *J Biol Chem* **263**:12163–12166 (1988).

8 Cundliffe E, How antibiotic-producing organisms avoid suicide. *Annu Rev Microbiol* **43**: 207–233 (1989).

9 Ueno Y, Nakajima M, Sakai K, Ishii K, Sato N and Shimada N, Comparative toxicology of trichothecene mycotoxins: inhibition of protein synthesis in animal cells. *J Biochem* (*Tokyo*) **74**: 285–296 (1973).

Parallel synthesis and automated data analysis: A versatile decision tool

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Abstract: The problems associated with the use of combinatorial chemistry in lead generation and optimization are discussed. A post-synthesis data evaluation process is described which can cope with large data sets from parallel synthesis effects. It relies on analysis of purity as well as on identification, and can efficiently limit compounds screened to manageable numbers.

Keywords: combinatorial chemistry; parallel synthesis; decision algorithm

Combinatorial chemistry recently emerged as a powerful tool for lead generation and lead optimization. Its growing importance is demonstrated by the observable fast implementation of combinatorial methodologies into all major pharmaceutical and agrochemical companies[1] and is backed by the desire for faster drug discovery cycles. Over the years a variety of combinatorial and automated synthesis techniques have been described.[2,3] Each of these has its own strengths and weaknesses with respect to the support of the various parts of the drug discovery process. For example split-and-mix methods[4,5] allow access to very large numbers of compounds for high-throughput screening, but the necessity for deconvolution and the risk of dealing with false positives are serious drawbacks. The latter is also true for the use of encoded libraries where the structure elucidation process of actives is significantly enhanced by an efficient read-out of the synthesis information encoded by some sort of tag.[6–8] Finally, parallel methods offer the possibility for fast single-compound synthesis,[9] but the process is labour-intensive and generally only a limited number of compounds can be reasonably handled at a time. Considerable advantages of parallel synthesis approaches are that no encoding other than keeping a spatial address in an array format is necessary and that compounds can be synthesized in any amount and by any chemistry either in solution or on a solid support.

Over recent years, improvements in laboratory robotics and data management tools have contributed significantly to the rapid growth of parallel synthesis methods. Some drawbacks in this approach still exist because it is inherent in the standard conditions usually applied that side-product formation or incomplete reaction occurs. However, screening impure products in a lead-optimization phase is undesired. Two solutions to avoid this problem exist: extensive reaction-optimization programs or application of rapid analysis and purification tecniques. Currently the latter strategy is of high interest[10] and in this paper our efforts concerning this topic are reported.

We favour an approach that deals with sample follow-up which is designed to yield pure compounds of known, as well as unknown structure. The thesis is that any novel chemical entity constitutes an element of molecular diversity and should be tested in a high-throughput random screen. To achieve this goal we rely on a two-step process which starts with an analytical LC/MS run of an aliquot of each sample followed by automatic data interpretation and sample categorization. Preparative purification is

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